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PUBLICATION DELAYED

Production of California Fish and Game was delayed for several months because of the lack of a state budget for the first two months of the fiscal year. We are behind schedule as you may have noticed. We hope to "catch-up" before the end of the year and appreciate your patience. Thank you.- *Eric R. Loft*

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POPULATION GENETIC STRUCTURE OF COHO SALMON *(ONCORHYNCHUS KISUTCH)* IN CALIFORNIA

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We used allozymes to examine the genetic structure of 27 populations of coho salmon from northern and central California. Genetic variability was low throughout the study area. Although 23 of 45 loci were variable, much of the observed variation was due to rare and uncommon alleles (frequency < 10%). Average heterozygosity estimates ranged from 0.000 to 0.050 with a mean of 0.027. We found little pattern in the distribution of variant alleles or genetic variation; we observed only weak associations between genetic identity and geographic location. Substantial transfers of coho salmon have taken place in California, and stocks of coho from Oregon have been introduced into the State. It is difficult to assess the relative influence of these stocking practices, selection, and random processes on the genetic structure of California coho salmon populations. The genetic structure of coho salmon in California, as well as current stocking practices, indicate that genetic stock identification of California coho salmon from individual streams or localized areas may be difficult with isozyme technology. Differences among coho salmon populations from the Pacific Northwest and California may allow genetic identification of stocks from broad geographic areas.

INTRODUCTION

Coho salmon are native to the west coast of North America from Monterey, California to Point Hope, Alaska (Scott and Crossman 1973). California populations of coho salmon have declined due primarily to habitat degradation associated with water diversions, mining, and deforestation (Netboy 1974). In order to manage and preserve California's coho salmon populations, basic information on genetic variability and gene flow in subpopulations and stocks will be essential (Allendorf and Utter 1979, Allendorf and Phelps 1981, Ihssen et al. 1981). As in other species of Pacific salmon, coho salmon make spawning migrations to their natal streams (Ricker 1972). The resulting restriction of gene flow among subpopulations spawning in different streams creates the potential for genetic differences to accumulate due to natural selection, genetic drift, and mutation (Wright 1943).

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Allozyme analyses have been used in research on salmonids of the Pacific Northwest to identify subpopulation structure (Utter, Allendorf, and Hodgins 1973, Kristianson and McIntyre 1976, Carl and Healy 1984, Wilmot and Burger 1985, Utter et al. 1989), to estimate levels of gene flow (Wehrhahn and Powell 1987, Berg and Gall 1988, Bartley and Gall 1990), to assess hatchery enhancement programs (Seeb, Seeb, and Utter 1986), to document interspecific hybridization (Bartley, Gall, and Bentley 1990), and to determine the composition of mixed fisheries (Pella and Milner 1987). However, coho salmon display the lowest level of allozyme variation of the five species of Pacific salmon (Allendorf and Utter 1979).

Here we report on the genetic structure of California populations of coho salmon. We sampled wild and hatchery stocks of fish, and analyzed their allozymes using standard laboratory techniques. Our main objective was to assess the use of allozymes of California coho salmon for stock identification purposes.

METHODS

Coho salmon were collected during the spring and summer of 1983-1985 and 1986 (Table 1, Fig. 1). Emigrating juvenile salmon were captured by standard backpack electro-shocking and fyke-netting. Salmon were either frozen on dry ice in the field or transported live to the laboratory. Samples of liver, muscle, blood, and eye were removed and frozen at -20°C.

Standard horizontal starch-gel electrophoresis and histo-chemical staining procedures were followed (Harris and Hopkinson 1976, Aebersold et al. 1987) to analyze 45 loci from 23 enzyme systems (Table 2). Allele nomenclature followed Allendorf and Utter (1979); a locus was considered polymorphic if we observed more than one allele in any collection. Loci that could not be scored in all samples were still included in the analyses.

Average heterozygosity, H, was calculated from the allele frequencies according to Nei (1973). Total gene diversity, H_T, was partitioned into within-sample, H_S, and between-sample, D_{ST}, components and the relative gene diversity, G_{ST}, was estimated (Nei 1973, Chakraborty and Leimar 1987). Deviations from Hardy-Weinberg proportions and gametic phase disequilibrium were assessed by the goodness-of-fit G-statistic (Sokal and Rohlf 1981) and Burrows' composite D (Weir 1979, Campton 1987), respectively. Duplicated loci, IDH-3,4, IDDH-1,2 and MDH-3,4 were omitted from the analyses of disequilibrium because variation could not be assigned to a particular locus. Homogeneity of allele frequencies among samples was tested by the G-test (Sokal and Rohlf 1981) after adjusting the significance level of $\alpha = 0.05$ for the number of simultaneous tests performed (Cooper 1968). The allele frequencies for samples from six geographic areas were pooled and again tested for homogeneity. The areas were (see Fig. 1 for sample number locations): 1) South Coast (sample numbers 1-2), 2) Russian River (3-5), 3) Mendocino Coast (6-18), 4) Eel River (19-21), 5) North Coast (22,23,27), and 6) Trinity River (24-26).

Genetic identities, I, based on allele frequencies of all 45 loci were calculated for each pair of samples (Nei 1978) and used to construct an unweighted pair-group

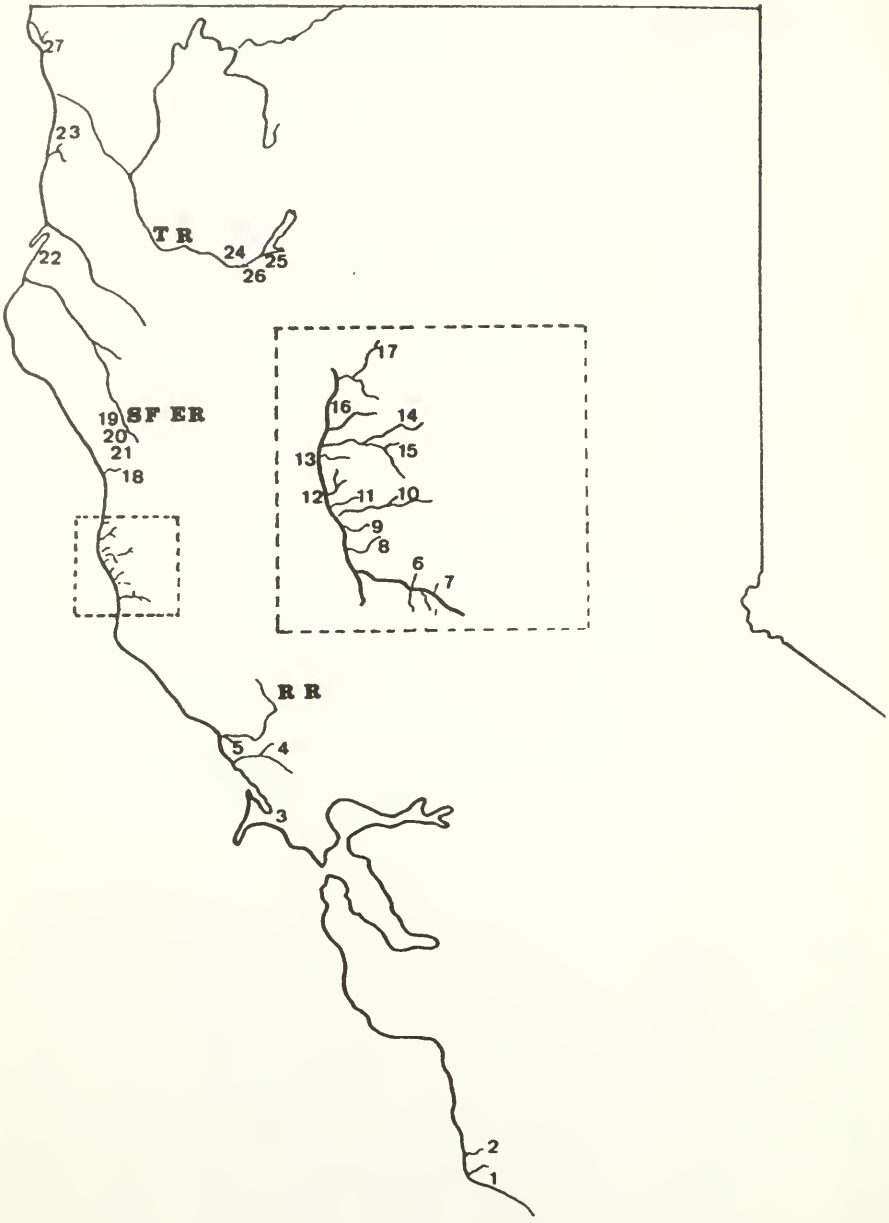

Figure 1. Collection sites for 27 populations of coho salmon in California. Numbers are identified in Table 1. TR = Trinity River, SF ER = South Fork Eel River, RR = Russian River.

Table 1. Collection sites for 27 populations of coho salmon from California. See Figure 1 for site locations. No. loci = number of loci analyzed. Heterozygosity is defined by Nei (1973).

Location (Site No.)	Number of fish	No. loci	Hetero-zygosity
Scott Creek (1)	39	35	0.000
Waddell Creek (2)	10	40	0.050
Lagunitas Creek (3)	32	35	0.024
Tanner Creek, Salmon Creek (4)	62	43	0.020
Willow Creek, Russian River (5)	38	33	0.014
Flynn Creek, Navarro River (6)	23	44	0.035
John Smith Creek, Navarro River (7)	15	42	0.034
Albion River (8)	30	45	0.038
Little River (9)	51	42	0.031
Twolog Creek, Big River (10)	23	44	0.042
Russian Gulch (11)	31	41	0.022
Caspar Creek (12)	82	45	0.034
Hare Creek (13)	28	44	0.033
Little North Fork Noyo River (14)	20	42	0.026
Kass Creek, Noyo River (15)	17	44	0.039
Pudding Creek (16)	47	44	0.032
Little N. Fk. Ten Mile R. (17)	22	45	0.026
Cottoneva Creek (18)	28	44	0.009
Huckleberry Cr., S.F. Eel R. (19)	52	44	0.042
Butler Creek, S.F. Eel R. (20)	30	44	0.026
Redwood Creek, S.F. Eel R. (21)	29	44	0.027
Elk River (22)	30	34	0.008
Prairie Creek (23)	3	43	0.042
Rush Creek, Trinity River (24)	7	32	0.014
Trinity River Hatchery (25)	111	44	0.039
Deadwood Creek, Trinity River (26)	26	40	0.008
West Branch Mill Cr., Smith R. (27)	30	39	0.016

dendrogram (Sneath and Sokal 1973). Gene diversity analysis followed Nei (1973).

A quantitative estimate of gene flow, the numbers of individuals exchanging genes between populations, was calculated from Wright's (1943) fixation index:

$$F_{ST} = 1/(4Nm+1)$$

where Nm is the average number of migrants per generation. This equation was solved for Nm by setting F_{ST} equal to the relative gene diversity G_{ST} (Nei 1977).

RESULTS

We observed allozyme variation at 23 of 45 (51%) isozyme loci studied (Appendices A-1 and A-2). Much of the variation was due to alleles with low frequencies. For example, 39% of the polymorphism observed at all loci over all samples involved variant alleles at frequencies of less than 5%. Also, of the 39 variant alleles identified, 27 (69%) occurred in three or fewer samples.

Table 2. Enzyme systems, abbreviations, number of loci scored, and tissue expression used in the analysis of 27 samples of coho salmon from northern and central California. M = muscle, E = eye, L = liver, B = blood.

Enzyme System	Abbreviation	No. of loci	Tissue
Aspartate aminotransferase	AAT	3	M,L
Aconitate hydratase	AH	1	L
Alcohol dehydrogenase	ADH	1	L
Adenylate kinase	AK	2	M,E
Fructose biphosphate aldolase	FBA	1	E
Creatine kinase	CK	5	M,E
ß-N-acetyl-D-galactosaminidase	ßGALA	1	L
Glycerol-3-phosphate dehydrogenase	GPDH	2	M
Glucose phosphate isomerase	GPI	3	M
L-iditol dehydrogenase	IDDH	2	L
Isocitrate dehydrogenase	IDH	4	M,E,L
Lactate dehydrogenase	LDH	5	M,E,L
Malate dehydrogenase	MDH	4	M,L
Mannose-6-phosphate isomerase	MPI	1	M,E,L
Phosphogluconate dehydrogenase	PGDH	1	M,L
Phosphoglycerate kinase	PGK	1	M,L
Phosphoglucomutase	PGM	2	M,L
Superoxide dismutase	SOD	1	L
Transferrin	TFN	1	B
Peptidase Substrates			
Glycyl-leucine	PEPA	1	M
	PEPC	1	E
Leucyl-glycyl-glycine	PEPB	1	M
Phenylalanyl-L-proline	PEPD	1	M

Genotypic frequencies conformed to Hardy-Weinberg proportions for all loci analyzed in all samples except for the PEPC locus in Flynn Creek (G = 4.60). Estimates of linkage disequilibrium revealed that 20 out of 299 (6.7%) comparisons significantly (P >.05) deviated from equilibrium values. No pattern of significant deviations was apparent: a pair of loci would be in disequilibrium in only one group and no more. The samples of coho salmon can be assumed to be in Hardy-Weinberg and linkage equilibrium, as the number of significant deviations was approximately what one would expect allowing for a 5% chance of a Type I statistical error.

Low levels of genetic variability were observed throughout the study area and only loose associations of alleles with geographic area were found. For example, the CK-2(85) allele was present at frequencies of 0.356 and 0.138 in two tributaries to the South Fork Eel River, Huckleberry Creek (19) and Redwood Creek (21), respectively, but the allele was absent from Butler Creek (20), which is also a tributary to the South Fork Eel River. The IDDH-1(150) allele was present in the three South Fork Eel River samples— Huckleberry Creek (19), Butler Creek (20), and Redwood Creek (21)— but was also found in Kass Creek (15) and Pudding Creek (16).

The GPI-3(85) allele was found exclusively in samples from the Trinity River system. The frequencies of this allele in Rush Creek (24), the Trinity River Salmon

Table 3. Summary of tests for homogeneity (*G* statistic, Sokal and Rohlf 1981) of allele frequencies for six geographic groups of California coho salmon (degrees of freedom in parentheses). Loci listed are those for which significant heterogeneity was observed before partitioning the data set into six geographic subgroups. N.V. and I.D. indicate no variation and insufficient data for comparisons, respectively.

Locus	South Coast	Russian River	Mendocino Coast	Eel River	North Coast	Trinity River
AAT-2	N.V.	N.V.	N.V.	N.V.	N.V.	0.98 (1)
CK-2	N.V.	N.V.	N.V.	30.68* (4)	N.V.	N.V.
BGALA	I.D.	N.V.	N.V.	2.17 (1)	2.49 (1)	N.V.
GPI-3	N.V.	N.V.	N.V.	N.V.	N.V.	5.14 (3)
IDDH-1	I.D.	I.D.	19.33 (12)	7.84	N.V.	I.D.
IDH-1	15.09* (1)	N.V.	N.V.	N.V.	N.V.	N.V.
IDH-2	27.90* (1)	N.V.	46.98* (9)	N.V.	5.67 (2)	N.V.
LDG-4	N.V.	8.33* (2)	N.V.	N.V.	10.38* (2)	N.V.
PGM-1	I.D.	4.28 (2)	84.06* (12)	1.40 (1)	4.61* (1)	N.V.
TFN	I.D	I.D.	76.95* (24)	9.53 (4)	I.D.	I.D.
PEPC	I.D.	I.D.	128.12* (13)	7.13* (2)	I.D.	0.98* (1)
PWPD	I.D.	I.D.	34.16* (14)	I.D.	I.D.	I.D.
TOTALS	42.99** (2)	12.62** (4)	389.60** (84)	58.75** (14)	23.15** (6)	12.11 (5)

* = $P < 0.05$.
** = significant *G* statistic after adjustment of α for multiple comparisons (Cooper 1968).

and Steelhead Hatchery (25), and Deadwood Creek (26) were 0.357, 0.126, and 0.135, respectively.

Heterozygosities ranged from 0.000 for Scott Creek (1) to 0.050 for Waddell Creek (2) (Table 1). The average heterozygosity of the 27 samples was 0.027. Geographic patterns or associations of heterozygosity estimates were not obvious; the two samples with the extreme values, Scott and Waddell creeks, were located adjacent to each other.

Total gene heterozygosity, H_T, and within-sample heterozygosity, H_S, of the 27 samples were 0.038 and 0.032, respectively. Genetic diversity among samples, D_{ST}, was 0.006. Thus, 84.2% of the total genetic variation (H_S/H_T) was due to variability within individual samples, whereas differences among samples ($G_{ST}=D_{ST}/H_T$) accounted for 15.8% of the variation. An estimate of 0.158 for G_{ST} yielded an estimate of 1.3 for *Nm*.

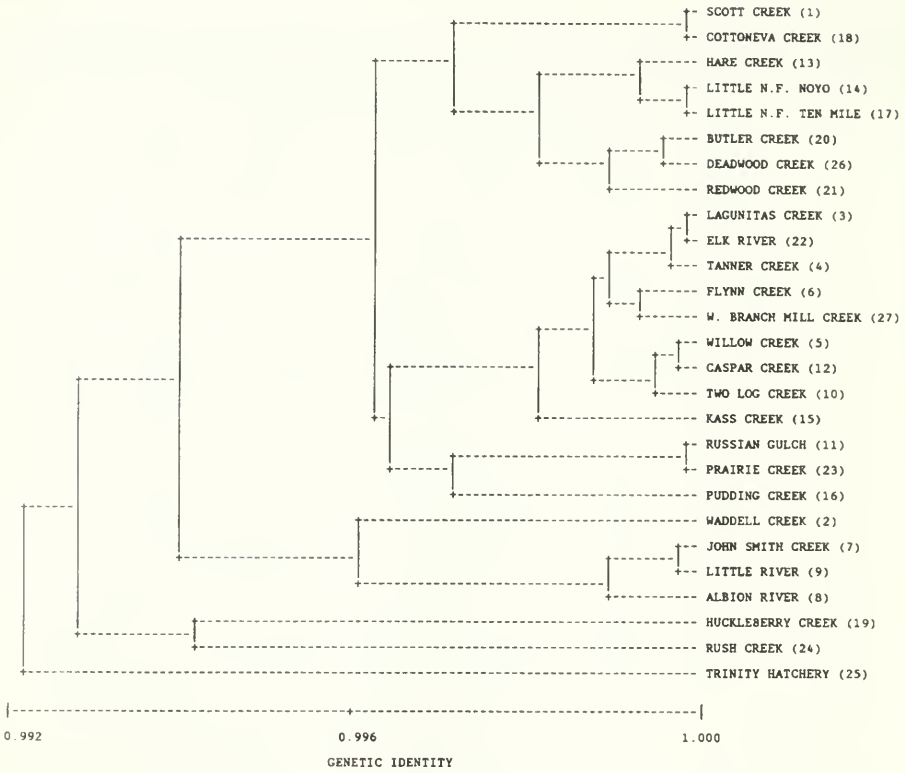

Figure 2. Dendrogram produced from unweighted pair group clustering of Nei's (1978) genetic identities between 27 populations of coho salmon from California.

Significant heterogeneity of allele frequencies was observed at 12 loci (Table 3) before partitioning the 27 samples into six geographic areas. After partitioning, heterogeneity still existed in all areas except for the Trinity River.

Average genetic identity between samples was 0.996. Various clusters of samples may appear in the dendrogram based on Nei's (1978) genetic identity values (Fig. 2). The clustering does not appear to reflect the geographic association of the samples.

DISCUSSION

The low level of genetic variability of California coho salmon (average heterozygosity value of 0.027) is characteristic of coho salmon from other areas of the Pacific Northwest. Allendorf and Utter (1979) reported an average heterozygosity of 0.015 for coho from Oregon and Washington; Olin (1981) found values of 0.026 to 0.052 (average 0.04) for populations from Oregon. Wehrhahn and Powell (1987) reported an average heterozygosity value of 0.0025 for Canadian populations of coho salmon, but they omitted the polymorphic transferrin locus.

Although 51% of the loci were polymorphic in this study, most of the variation was due to rare alleles that occurred in only a few samples. Wehrhahn and Powell (1987) found no loci that were polymorphic in more than one-fifth of 96 Canadian coho salmon populations. Olin (1984) found 31 of 53 (58%) loci to be polymorphic in 23 samples of Oregon coho salmon. In Olin's samples, 78% of the variant allele frequencies were less than 0.10. For coho populations from northwestern Washington, Reisenbichler and Phelps (1987) found only 2 of 54 loci (4%) with a common allele frequency of < 0.95.

The excessive and often undocumented transplants of coho salmon throughout the Pacific Northwest may obscure natural patterns of genetic variability and make geographical identification of stocks difficult. In the 1950s and 1960s the California Department of Fish and Game (CDFG) imported coho salmon eggs from Oregon to start most of California's hatchery stocks. During this same period, an extensive coho salmon stocking effort was undertaken in coastal streams using yearling fish produced from Pudding Creek and Noyo River egg sources. About 500,000 yearling coho salmon were stocked annually into watersheds of the north and central coastal regions. The Trinity River Salmon and Steelhead Hatchery coho program was started with eggs from native fish, as well as those from the Eel River, the Cascade and Alsea hatcheries in Oregon and the Noyo River Egg Taking Station. Currently, the Mad River and Warm Springs hatcheries provide fish and eggs for some coastal waters, as well as for the State's cooperative rearing programs. Eggs from the Noyo River Egg Taking Station are still taken for distribution of yearling coho salmon to other coastal waters, and State facilities routinely exchange coho eggs, unless there is a concern for disease transmission (L. Boydstun, Calif. Dept. Fish and Game, pers. comm.).

The coho population in Waddell Creek, Santa Cruz County, has apparently received eggs and transplants from hatcheries in northern California and Oregon and from private aquaculturists that did not keep records of their egg sources (D. Streig, Monterey Bay Salmon and Trout Project, pers. comm.). Thus, the varied origin of coho salmon in Waddell Creek may explain its high level of heterozygosity.

It is interesting that Scott Creek, which is about 6 km from Waddell Creek, showed no genetic variation. This suggests that coho salmon populations from the two creeks are maintaining some degree of reproductive isolation. Shapovalov and Taft (1954) observed that about 15% of the salmon tagged from the wild run of coho salmon in Waddell Creek strayed into Scott Creek, whereas about 27% of the fish marked in Scott Creek strayed into Waddell Creek. This amount of straying should be sufficient to homogenize the gene pools between the two creeks, if the straying salmon are actually contributing to the spawn (Allendorf and Phelps 1981), and unless Waddell Creek is currently being infused with new genes.

Although we could define very little in the way of a geographic pattern to the distribution of variant alleles, we did observe significant allele frequency differences among the 27 samples, which suggests that the coho salmon gene pool is not homogeneous throughout California. Given past and present stocking practices, this result is somewhat surprising. The causes of this heterogeneity are at present unclear. We do not know the relative influences of selection, drift, sampling error and human

activities on the genetic structure of California coho.

Some allele frequency differences may be maintained by selection. Certain transferrin genotypes have been shown to have increased resistance to bacterial kidney disease in specific stocks of coho salmon (Suzumoto et al. 1977, Winter, Schreck, and McIntyre 1980, Withler and Evelyn 1990). These same genotypes were also shown to be associated with poor growth and survival in one hatchery (McIntyre and Johnson 1977).

The frequency of transferrin alleles has been shown to vary significantly within the range of coho salmon (Utter, Ames, and Hodgins 1970). A north-south cline in the frequency of the TFN-(103) allele exists between samples from California and Oregon (Fig. 3); average frequencies of the allele in Olin's (1984) collections and ours were 0.34 and 0.80, respectively. Oregon also has had an extensive program of stock transfers within the State (Olin 1984). The fact that this cline exists, in spite of the homogenizing effects of stock transfers, may indicate a selective advantage for certain transferrin genotypes in California.

Transferrin expression on starch gels requires that the plasma be extracted from fresh blood and frozen prior to electrophoresis. Unfortunately, we were unable to score this locus for many of our collections because whole blood was frozen. With incomplete allele frequency data and vague stocking records, it is difficult to

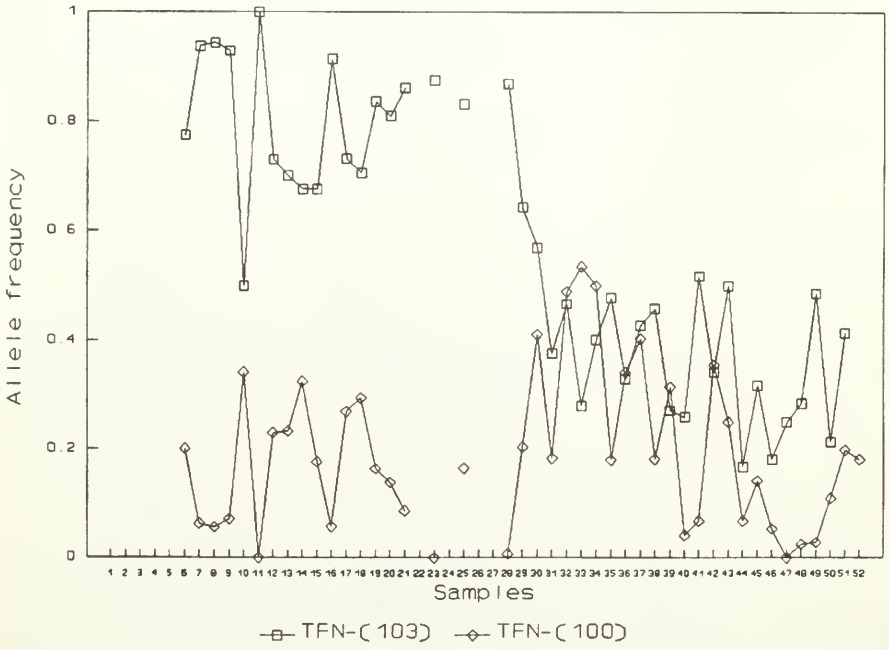

Figure 3. Frequencies of the TFN-(103) (squares) and TFN-(100) (diamonds) alleles in 27 samples of California coho salmon and 25 samples of Oregon coho salmon. Sample numbers 1-27 are from present study and numbers 26-52 are from Olin (1984). Sample numbers arranged from south to north with number 1 the most southern sample and number 52 the most northern. Gaps in line drawing indicate no data.

determine accurately the causes of the distribution of this allele.

Coho salmon may not be genetically differentiated on a geographic basis within their range in California, but may show differentiation when examined on a coast-wide basis. For example, Olin (1984) observed variant alleles at AAT-2, AH, CK-2, CK-3, PEPC and GPI-2, that were either extremely rare or not present in our California collections. The north-south cline in TFN-(103) may also serve to delineate broad areas of coho salmon. In addition, a GPI-3 variant we found in the Trinity River was only observed in one Oregon sample. Wehrhahn and Powell (1987) found geographical differences among populations of coho salmon from Vancouver Island, British Columbia, and Oregon. They felt that genetic differentiation may have resulted from bottlenecks and founder effects associated with glaciation in the late Pleistocene.

The natural genetic structure of coho salmon populations is assumed to be maintained by a balance between selection and gene flow. The level of gene flow will depend on the salmon's homing ability and the tendency to stray into non-natal streams. In coastal streams, high levels of straying and gene flow are expected as salmon from small unstable streams were shown to home less precisely than salmon from large stable streams (Quinn 1982). In our study, the average level of gene flow among coho salmon samples was considered high ($Nm = 1.3$), from an evolutionary standpoint (Slatkin 1981), but low from a population genetic standpoint (Allendorf and Phelps 1981).

Because coho salmon in California are restricted to the smaller, unstable coastal streams (Moyle 1976), straying may play a large role in influencing genetic variation within our study area. Berg and Gall (1988) also reported a lack of a genetic/geographic association among collections of coastal rainbow trout, *O. mykiss*, from northern California. Whereas, others (Utter et al. 1989, Bartley and Gall 1990) observed a strong geographic component to the genetic structure of chinook salmon, *O. tshawytscha*, a species that does inhabit large rivers in California and elsewhere.

An important application of allozyme analysis of salmonids has been in providing a means for stock identification to fishery scientists and managers (Pella and Miller 1987, Brodziak et al. in press). The application of these techniques to differentiate coho salmon populations within California appears problematic given the nature of the allozyme variation observed here. The usefulness of genetic stock identification for fishery management depends on the level of genetic divergence of populations, as well as on a fairly constant genetic structure of baseline populations: past and present transfers of coho salmon in California may have rendered the technique inappropriate for identifying stocks from localized areas in California. Significant allele frequency heterogeneity exists within the larger geographic areas except for the Trinity River area, and these geographic areas are not reflected in the dendrogram of genetic similarities.

We recommend continued study of California coho salmon populations. Genetic analyses could be greatly improved by increasing the samples sizes; only the sample from the Trinity River Salmon and Steelhead Hatchery contained the recommended number of 100 fish currently used by laboratories involved with salmon genetic stock

identification. Thorough examination of CDFG stocking records would also be beneficial in determining the possible existence of native gene pools or evaluating the effects of selection and random processes on coho salmon genetic structure. Improvements in estimation procedures, the increased number of isozyme loci being analyzed, and new techniques for DNA-level analyses (Hallerman and Beckman 1988) may also increase the usefulness of genetic analyses in stock identification of California coho salmon.

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Appendix A-1. Allele frequencies at the AAT-2 through IDH-3 loci from 27 populations of California coho salmon. See Figure 1 for stream locations by sample site number (shown under each stream name).

Locus	(allele)	*Scott Creek* 1	*Waddell Creek* 2	*Lagunitas Creek* 3	*Tanner Creek* 4	*Willow Creek* 5	*Flynn Creek* 6	*John Smith Creek* 7	*Albion River* 8	*Little River* 9	*Twolog Creek* 10	*Russian Gulch* 11	*Caspar Creek* 12	*Hare Creek* 13	*Little N.F. Noyo River* 14
AAT-2	(100)		1.000		1.000		1.000	1.000	1.000	1.000	1.000	1.000	0.994	1.000	1.000
	(110)												0.006		
AAT-3	(100)		1.000		1.000		1.000	1.000	1.000		0.977		1.000	1.000	1.000
	(120)										0.023				
AH	(100)	1.000		0.969	1.000	1.000	1.000		1.000	1.000	1.000	1.000	1.000	1.000	1.000
	(125)			0.016											
	(112)			0.016											
CK-2	(100)	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	(85)														
CK-3	(100)	1.000	0.800	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.994	1.000	1.000
	(110)		0.200										0.006		
BGALA	(100)		1.000		1.000	1.000	1.000	1.000	1.000	1.000	1.000		1.000	1.000	1.000
	(90)														
GPI-2	(100)	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	(150)														
	(50)														
GPI-3	(100)	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.967	0.980	1.000	1.000	0.988	1.000	1.000
	(115)														
	(85)														
	(NULL)								0.033	0.020			0.012		
IDDH-1	(100)		1.000		1.000		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	(150)														
IDH-1	(100)	1.000	0.750	1.000	1.000		1.000		1.000		1.000		0.992	1.000	1.000
	(120)		0.250										0.008		
IDH-2	(100)	1.000	0.563	1.000	1.000		0.913		0.591		0.913		0.873	0.850	1.000
	(80)		0.438				0.087		0.409		0.087		0.127	0.150	
IDH-3	(100)	1.000	1.000	1.000	1.000	1.000	0.978	1.000	1.000	1.000	0.957	1.000	0.988	1.000	1.000
	(150)										0.022				
	(130)						0.022						0.013		
	(120)										0.022				

Appendix A-1. (Continued)

Locus	(allele)	Kass Creek 15	Pudding Creek 16	Little N.F. Ten Mile R. 17	Cottoneva Creek 18	Huckleberry Creek 19	Butler Creek 20	Redwood Creek 21	Elk River 22	Prairie Creek 23	Rush Creek 24	Trinity Hatchery 25	Deadwood Creek 26	West Branch Mill Creek 27
AAT-2	(100)	1.000	1.000	1.000	1.000	1.000	1.000	0.983		1.000		0.865	1.000	
	(110)							0.017				0.135		
AAT-3	(100)	0.912	0.978	1.000	1.000	1.000	1.000	1.000		1.000		1.000	1.000	
	(120)	0.088	0.022											
AH	(100)	1.000	0.826	1.000	1.000	1.000	1.000	1.000	1.000		1.000	1.000	1.000	1.000
	(125)		0.174											
	(112)													
CK-2	(100)	1.000	1.000	1.000	1.000	0.644	1.000	0.862	1.000	1.000		1.000	1.000	1.000
	(85)					0.356		0.138						
CK-3	(100)	1.000	1.000	1.000	1.000	1.000	1.000	0.983	1.000	1.000		1.000	1.000	1.000
	(110)							0.017						
βGALA	(100)	1.000	1.000	1.000	1.000	1.000	1.000	1.000		0.625		1.000	1.000	0.867
	(90)									0.375				0.133
GPI-2	(100)	1.000	1.000	1.000	1.000	0.981	0.983	1.000	0.967	0.875	1.000	0.991	1.000	1.000
	(150)					0.019	0.017		0.033	0.125				
	(50)											0.009		
GPI-3	(100)	1.000	1.000	1.000	1.000	1.000	1.000	0.983	1.000	1.000	0.643	0.874	0.865	1.000
	(115)							0.017						
	(85)										0.357	0.126	0.135	
	(NULL)													
IODH-1	(100)	0.912	0.989	1.000	1.000	0.940	0.948	0.931	1.000	1.000		1.000		1.000
	(150)	0.088	0.011			0.060	0.052	0.069						
IDH-1	(100)	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000		1.000	1.000	1.000
	(120)													
IDH-2	(100)	0.971	0.913	1.000	1.000	1.000	1.000	1.000	1.000	0.875		1.000	1.000	1.000
	(80)	0.029	0.087							0.125				
IDH-3	(100)	0.941	1.000	1.000	1.000	0.952	0.983	1.000	1.000	1.000	1.000	0.621	0.981	1.000
	(150)	0.029										0.379		
	(130)	0.029				0.048	0.017						0.019	
	(120)													

Appendix A-2. Allele frequencies at the IDH-4 through PEPD loci from 27 populations of California coho salmon. See Figure 1 for stream locations by sample site number (shown under each stream name).

Locus (allele)		Scott Creek 1	Waddell Creek 2	Lagunitas Creek 3	Tanner Creek 4	Willow Creek 5	Flynn Creek 6	John Smith Creek 7	Albion River 8	Little River 9	Twolog Creek 10	Russian Gulch 11	Caspar Creek 12	Hare Creek 13	Little N.F. Noyo River 14
IDH-4	(100)	1.000	0.750	0.968	0.984	0.961	1.000	1.000	1.000	1.000	0.978	1.000	1.000	1.000	1.000
	(120)					0.039									
	(90)										0.022				
	(70)		0.250	0.032	0.016										
LDH-3	(100)		1.000		1.000		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	(125)														
LDH-4	(100)	1.000	1.000	0.871	0.893	0.987	1.000	1.000	1.000	1.000	1.000	1.000	0.994	1.000	1.000
	(115)			0.129	0.105	0.013							0.006		
MDH-3	(100)	1.000	0.700	0.922	0.893	0.986	0.978	0.714	0.983	0.902	0.957	0.952	0.982	1.000	1.000
	(110)														
	(120)		0.300	0.078	0.107	0.014	0.022	0.286	0.017	0.098	0.043	0.048	0.018		
MPI	(100)		1.000		1.000		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	(110)														
	(80)														
PGDH	(100)		1.000	1.000	1.000		1.000	1.000	1.000	0.949	1.000	1.000	1.000	1.000	
	(120)									0.051					
PGM-1	(100)	1.000		0.760	0.871	0.892	0.682	0.700	0.767	0.630	0.804	0.887	0.846	0.786	0.950
	(120)			0.240	0.129	0.108	0.318	0.300	0.233	0.370	0.196	0.113	0.156	0.214	0.050
TFN	(103)						0.775	0.938	0.944	0.929	0.500	1.000	0.730	0.700	0.676
	(100)						0.200	0.063	0.056	0.071	0.341		0.230	0.233	0.324
	(97)						0.025				0.159		0.041	0.067	
	(106)														
PEPA	(100)	1.000		1.000	1.000	0.917	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.925
	(120)					0.083									
	(110)														
	(89)														0.075
PEPC	(100)	1.000					0.455	0.615	0.667	0.720	0.523	0.758	0.513	0.768	0.700
	(80)						0.545	0.346	0.300	0.280	0.477	0.242	0.487	0.232	0.275
	(120)							0.038	0.033						0.025
PEPD	(100)		1.000		1.000			1.000	0.900	1.000		0.850	0.991	0.981	
	(120)											0.150	0.009	0.019	
	(90)								0.100						

Appendix A-2. (Continued)

Locus (allele)		Kass Creek 15	Pudding Creek 16	Little N.F. Ten Mile R. 17	Cottoneva Creek 18	Huckleberry Creek 19	Butler Creek 20	Redwood Creek 21	Elk River 22	Prairie Creek 23	Rush Creek 24	Trinity Hatchery 25	Deadwood Creek 26	West Branch Mill Creek 27
IDH-4	(100)	1.000	0.967	1.000	1.000	0.962	1.000	1.000	1.000	0.875	1.000	1.000	1.000	1.000
	(120)					0.019								
	(90)					0.019								
	(70)		0.033							0.125				
LDH-3	(100)	0.971	0.989	1.000	1.000	1.000	1.000	0.966		1.000	1.000	1.000	1.000	1.000
	(125)	0.029	0.011					0.034						
LDH-4	(100)	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.883	1.000	1.000	0.970	1.000	1.000
	(115)													
MDH-3	(100)	0.912	1.000	1.000	1.000	0.923	0.900	0.966	1.000	1.000	1.000	1.000		1.000
	(110)							0.034						
	(120)	0.088				0.077	0.100							
MPI	(100)	1.000	1.000	1.000	1.000	1.000	1.000	1.000		1.000		0.924	1.000	1.000
	(110)											0.061		
	(80)											0.015		
PGDH	(100)	1.000	0.964	1.000		1.000	1.000	1.000	1.000	1.000		1.000	1.000	1.000
	(120)		0.036											
PGM-1	(100)	0.941	0.968	0.841	1.000	0.990	0.983	1.000		1.000		1.000	1.000	0.732
	(120)	0.059	0.032	0.159		0.010	0.017							0.268
TFN	(103)	0.676	0.914	0.731	0.706	0.837	0.810	0.862		0.875		0.832		
	(100)	0.176	0.057	0.269	0.294	0.163	0.138	0.086				0.164		
	(97)	0.147	0.029				0.052	0.052		0.125				
	(106)											0.005		
PEPA	(100)	1.000	0.989	0.864	1.000	0.952	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	(120)													
	(110)					0.019								
	(89)		0.011	0.136		0.029								
PEPC	(100)	0.265	0.500	0.841	1.000	0.827	0.638	0.776		0.625	1.000	0.832		
	(80)	0.735	0.500	0.159		0.173	0.362	0.224		0.375		0.168		
	(120)													
PEPD	(100)			1.000	1.000						1.000		0.978	
	(120)													
	(90)												0.022	

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CULTURE OF SPOTTED SEATROUT, ORANGEMOUTH CORVINA, AND THEIR HYBRIDS

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Artificial propagation research with spotted seatrout (*Cynoscion nebulosus*) and orangemouth corvina (*Cynoscion xanthulus*) prompted interest in intrageneric hybrids between the two species. Spotted seatrout and hybrid fry were produced utilizing strip-spawning procedures following injection with human chorionic gonadotropin. Orangemouth corvina fry were obtained from use of a combination of hormone (des-Gly$_{10}$ [D-Ala^6]-luteinizing hormone-releasing hormone [1-9] ethylamide [LHRHa] and pimozide) and photoperiod-temperature manipulation to induce spawns. Twenty-two pond culture trials ranging from 18 to 40 days were conducted from 1984 to 1986. Fingerling survival for spotted seatrout, orangemouth corvina, spotted seatrout female X orangemouth corvina male, and reciprocal hybrids averaged (± SD) 51 ± 25.1% (*n*=7), 44 ± 39.7% (*n*=8), 36 ± 7.0% (*n*=5), and 76 ± 33.9% (*n*=2), respectively. Respective average production for the four fishes was 1.1, 2.9, 1.1, and 0.7 kg/ha/day. Average total lengths and weights were 44 mm and 0.74 g for spotted seatrout; 36 mm and 0.41 g for orangemouth corvina; 50 mm and 1.03 g for spotted seatrout X orangemouth corvina hybrids; and 48 mm and 1.43 g for reciprocal hybrids. Numbers of fingerlings reared were: spotted seatrout - 68,000; orangemouth corvina - 835,900; spotted seatrout female X orangemouth corvina male hybrids - 42,400; and reciprocal hybrids - 25,600. These successful initial attempts at pond culture of orangemouth corvina and the two hybrids indicate the fish can be produced in large numbers to satisfy requirements of a management or population enhancement stocking program.

INTRODUCTION

Spotted seatrout (*Cynoscion nebulosus*) support important recreational fisheries along the southeastern Atlantic and Gulf coasts (Perret et al. 1980, Collins 1981). Similarly, the orangemouth corvina (*Cynoscion xanthulus*) is a valued game fish in the Gulf of California and the Salton Sea (Whitney 1961, Black 1974). Both species are predatory (Whitney 1961, Perret et al. 1980) and therefore candidates for stocking in inland reservoirs overpopulated with forage species. Orangemouth corvina readily adapt to freshwater (Prentice 1985) but die at temperatures below 12°C in freshwater.

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Conversely, spotted seatrout do not adapt well to freshwater but tolerate temperatures down to 3°C (Texas Parks and Wildlife Department, unpublished data). Artificial propagation research with both fishes (Colura 1974, Arnold et al. 1976, Colura et al. 1976, Porter and Maciorowski 1984, Colura et al. 1988, Prentice et al. 1989) prompted interest in intrageneric hybrids between the two species. A hybrid between the two fish might exhibit the wide range in salinity tolerance of the orangemouth corvina and the lower temperature tolerance of the spotted seatrout, providing a fish suitable for stocking in reservoirs. Accordingly, a program was undertaken to determine if spotted seatrout and orangemouth corvina could be hybridized and cultured in saltwater ponds. The present report provides a description of fry and fingerling production of the two hybrids and the parent species.

MATERIALS AND METHODS

Spawning and Fry Culture

Orangemouth corvina were collected as subadults from the Salton Sea (California) in 1981 and 1985, and transported to the Texas Parks and Wildlife Department, Heart of Hills Research Station (HHRS), Ingram, Texas, and the Perry R. Bass Marine Fisheries Research Station (MFRS), Palacios, Texas. Fish were reared to adult size in indoor recirculating seawater systems and outdoor saltwater ponds (Prentice et al. 1989). Spotted seatrout were captured by hook and line from Matagorda Bay (Texas) and transported to the MFRS. Upon arrival, spotted seatrout were biopsied (females) or abdominally massaged (males) to determine gonadal condition as described by Colura (1974).

Gametes for hybridization were obtained by photoperiod and temperature conditioning, and hormone induction techniques. Male orangemouth corvina were brought to spawning condition by a previously described temperature-photoperiod regime (Prentice et al. 1989). Female spotted seatrout were intramuscularly injected with 100 mg/kg human chorionic gonadotropin (HCG) the morning following capture to induce ovulation. For female orangemouth corvina X male spotted seatrout (OMC X SST) hybridization attempts (27 June and 28 August 1986), females maintained in outdoor earthen ponds were captured by hook and line and examined by intraovarian biopsy (Hoff et al. 1972) to determine eligibility for hormone therapy. Mean oocyte diameter of greater than 0.45 mm was used as the criteria for determining eligibility for hormone administration (Colura et al. 1988). The day before strip-spawning, females were injected with 50 mg/kg HCG. Hormone administration procedures for production of spotted seatrout were similar to those described for spotted seatrout females during hybridization attempts. Strip-spawning techniques for orangemouth corvina and spotted seatrout to produce hybrids and spotted seatrout were similar to those described by Colura (1974) for spotted seatrout. Females of both species ovulated 26-32 hours after HCG injection, and eggs were fertilized by the dry method (Davis 1953).

Fertilized orangemouth corvina eggs were obtained from hormone-induced spawning at the HHRS (28 and 29 April 1986) and four spawns at the MFRS (16 June,

17 July, 9 August, and 20 August 1986). For the former, female orangemouth corvina at the HHRS were injected with a mixture of 0.2 mg/kg LHRHa and 10 mg/kg pimozide, followed by a second injection of 0.1 mg/kg LHRHa 48 hours later (Prentice and Thomas 1987). Fish were subsequently allowed to spawn in the holding tanks, eggs were collected after spawning was completed. Fertilized eggs spawned at the HHRS were transported to the MFRS for hatching and pond rearing. Spawns at the MFRS were induced by abruptly decreasing tank temperatures 2-5°C during the summer portion of a previously described temperature-photoperiod regime (Prentice et al. 1989).

The number of eggs produced per strip-spawned female or recovered from the spawning tank was determined by volumetric estimation (Bayless 1972). At least 2 hours after spawning, three samples of approximately 100 eggs from each spawn were microscopically examined for cell cleavage to estimate percent fertilization. Fertilized eggs were incubated in a 1900-l cone-bottomed fiberglass tank at 24-29°C and 27-29 o/oo salinity through hatching. Digestive tract development for all fishes was complete approximately 2.5 days after spawning, at which time fry were concentrated and fry numbers determined by volumetric estimation (Bayless 1972). After enumeration, fry were stocked into prepared earthen ponds.

Fingerling Culture

Twenty-two pond culture trials were performed using 0.2, 0.4, and 0.8-ha rectangular earthen ponds at the MFRS. Ponds were filled with 0.5-mm mesh filtered Matagorda Bay water using the puddle technique of Bonn et al. (1976) 7-30 days before introducing fry. Fry stocking rates ranged from 4,700 to 1,365,000 fry/ha and were determined by fry availability (Table 1). All ponds were fertilized with cottonseed meal, phosphoric acid (P_2O_5), and urea, but specific fertilization rates differed (Table 2). Half of the cottonseed meal scheduled for application was initially applied to dry pond bottoms, liquid phosphoric acid and granular urea were added to partially filled ponds. Ponds were filled to a depth of 1.5-2.0 m, and remaining cottonseed meal was broadcast over the pond three times weekly in equal allotments to maintain zooplankton. Application of cottonseed meal generally continued for 3 weeks after fry stocking. The different cottonseed meal application rates (Table 2) reflect different time intervals between initial filling and fry stocking, number of days in production, and adjustments necessary to maintain adequate dissolved oxygen. The elevated inorganic fertilization rates for trials 15-22 reflect a decision to increase the amount and frequency of phosphoric acid and urea application in 1986. For trials 15-22, one half of the total amount of phosphoric acid and urea was applied to partially filled ponds. The remaining inorganic fertilizer was applied to the ponds in two equal applications 10 and 20 days after filling was completed.

Zooplankton from each pond were sampled with a flexible impeller pump apparatus (Farquhar and Geiger 1984) three times weekly. Twenty-five liters of water were collected at the pond drain box and passed through a 63-µm Wisconsin plankton net. Organisms were identified by microscopy and enumerated using standard

Table 1. Stocking and production summary and mean water temperature (± SD) of spotted seatrout, spotted seatrout female X orangemouth corvina male (SST X OMC) hybrid, orangemouth corvina, and orangemouth corvina female X spotted seatrout male (OMC X SST) hybrid saltwater pond culture trials.

Trial	Group	Stocking date	Days in pond	Stock. rate (fry/ha)	Survival (%)	Production (kg/ha/day)	Mean water Temp. (°C)
1	SST X OMC	23 Jun 1984	32	95,000	41.8	1.20	28 ± 0.7
2	SST X OMC	19 May 1985	31	70,000	33.6	0.87	26 ± 1.5
3	Seatrout	19 May	31	>70,000	>100	1.68	27 ± 1.5
4	Seatrout	27 May	31	70,000	26.4	0.66	26 ± 1.4
5	SST X OMC	27 May	31	45,000	26.7	0.58	27 ± 1.4
6	Seatrout	27 May	31	45,000	35.6	0.79	27 ± 1.4
7	Seatrout	27 May	31	45,000	41.9	0.63	26 ± 1.4
8	SST X OMC	27 Jul	31	129,000	31.8	1.37	29 ± 0.5
9	SST X OMC	27 Jul	31	129,000	43.4	1.41	29 ± 0.5
10	Seatrout	27 Jul	31	129,000	44.6	1.28	29 ± 0.5
11	Seatrout	27 Jul	31	129,000	67.1	1.51	29 ± 0.5
12	Seatrout	27 Jul	31	129,000	41.9	0.87	29 ± 0.5
13	Corvina	29 Sep	40	69,000	9.4	0.11	22 ± 3.9
14	Corvina	29 Sep	40	69,000	23.2	0.28	21 ± 5.8
15	Corvina	01 May 1986	29	35,800	16.6	0.18	24 ± 1.6
16	Corvina	18 Jun	28	133,800	0	0	29 ± 0.5
17	Corvina	17 Jul	27	729,800	37.8	2.45	29 ± 0.4
18	Corvina	11 Aug	27	195,000	95.6	3.20	28 ± 1.5
19	Corvina	21 Aug	22	1,354,000	64.2	8.30	27 ± 1.2
20	Corvina	21 Aug	22	>1,365,000	>100	8.50	28 ± 1.3
21	OMC X SST	29 Jun	27	4,700	52.0	0.25	29 ± 0.5
22	OMC X SST	01 Sep	18	>71,500	>100	1.10	27 ± 0.9

subsampling and counting techniques (Weber 1973, APHA et al. 1985).

Pond culture periods ranged from 18 to 40 days (Table 1). At harvest, 100 randomly selected fingerlings were sampled from each pond. Standard length (SL), total length (TL), and weight were determined for each specimen. Remaining fish from each pond were mass weighed with a dairy scale. Total number of fish recovered was determined by dividing the mean individual weight of 100 fish into the weight of fish harvested.

Water quality determinations were performed daily at each pond drain box between sunrise and 0830 hours. Dissolved oxygen and temperature were determined using a dissolved oxygen meter and a thermometer, respectively. Salinity was measured by a refractometer or salinity meter. Water in ponds was exchanged when dissolved oxygen concentrations fell below 3.0 mg/l during the night.

All statistical comparisons of fish production and size were limited to equal size ponds stocked at the same rate and at the same time. A *t*-test was used to test for differences in production, SL, TL, weight, condition factor and arcsine transformed

Table 2. Summary of fertilizer application and mean (± SD) zooplankton density for spotted seatrout female X orangemouth corvina male (SST X OMC) hybrid, spotted seatrout, orangemouth corvina, and orangemouth corvina female X spotted seatrout male (OMC X SST) hybrid pond culture trials.

Trial	Group	Pond size (ha)	Initial filling	CSM[a] (kg/ha)	P_2O_5 (l/ha)	Urea (kg/ha)	Stocking date	Total zooplankton density (No./l)
1	SST X OMC	0.4	08 Jun 1984	570	9.9	4.6	23 Jun 1984	2169 ± 959
2	SST X OMC	0.2	06 May 1985	475	3.8	1.7	19 May 1985	1189 ± 1253
3	Seatrout	0.2	06 May	475	3.8	1.7	19 May	344 ± 233
4	Seatrout	0.2	06 May	475	3.8	1.7	27 May	358 ± 260
5	SST X OMC	0.2	06 May	506	3.8	1.7	27 May	524 ± 363
6	Seatrout	0.2	06 May	506	3.8	1.7	27 May	763 ± 651
7	Seatrout	0.2	06 May	506	3.8	1.7	27 May	469 ± 249
8	SST X OMC	0.2	12 Jul	697	3.8	1.7	27 Jul	1614 ± 1654
9	SST X OMC	0.2	12 Jul	697	3.8	1.7	27 Jul	2356 ± 1882
10	Seatrout	0.2	12 Jul	697	3.8	1.7	27 Jul	2001 ± 1729
11	Seatrout	0.2	12 Jul	697	3.8	1.7	27 Jul	2239 ± 1837
12	Seatrout	0.2	12 Jul	697	3.8	1.7	27 Jul	2536 ± 1935
13	Corvina	0.2	17 Sep	793	3.8	1.7	29 Sep	79 ± 59
14	Corvina	0.2	17 Sep	793	3.8	1.7	29 Sep	109 ± 72
15	Corvina	0.8	14 Apr 1986	644	21.3	20.5	01 May 1986	686 ± 413
16	Corvina	0.8	19 May	611	27.3	28.5	18 Jun	149 ± 42
17	Corvina	0.8	08 Jul	412	18.1	18.9	17 Jul	548 ± 310
18	Corvina	0.8	07 Aug	504	14.6	6.9	11 Aug	957 ± 588
19	Corvina	0.2	15 Aug	540	17.5	8.0	21 Aug	1001 ± 1329
20	Corvina	0.2	15 Aug	600	17.5	8.0	21 Aug	797 ± 205
21	OMC X SST	0.8	18 Jun	442	27.1	28.1	29 Jun	1200 ± 904
22	OMC X SST	0.2	25 Aug	508	17.5	8.0	01 Sep	781 ± 521

[a]CSM = Cottonseed Meal

survival of spotted seatrout and SST X OMC hybrids cultured under similar conditions (pond trials 8-12). A *t*-test was also used to test for differences in SL, TL, weight, and condition factor of SST X OMC from one trial and spotted seatrout from two trials (trials 5-7) conducted in similar size ponds over the same time period.

RESULTS

Spawning and Fry Culture

Egg fertilization for all spawns ranged from 18 to 85%, although fertilization for the SST X OMC hybrid was approximately half that of the parent species or the reciprocal hybrid (Table 3). Fry survival at 2 days ranged from <1% to 57%. Orangemouth corvina fry survival averaged 49% for photoperiod and temperature induced spawns in contrast to < 5% for hormone induced spawns. Spotted seatrout

Table 3. Summary of spawning data and fry yields for spotted seatrout, orangemouth corvina, and their hybrids.

Species	Spawning date	No. females	Total eggs	Viable eggs	Fertilization (%)	No. fry at 2 days	Survival (%)
SST X OMC	20 May 1984	2	253,400	71,700	28.28	38,000	53.00
	16 May 1985	5	2,703,000	489,000	18.09	14,200	3.44
	24 May	2	1,158,400	355,000	30.65	9,000	2.53
	25 Jul	4	1,038,100	534,300	51.45	51,700	9.68
	Total	13	5,152,900	1,450,000	28.14	112,900	7.78
Seatrout	16 May 1985	9	4,445,000	3,150,000	70.70	784,000	24.89
	24 May	10	1,860,000	517,000	27.80	125,000	24.17
	25 Jul	28	3,302,000	1,600,000	48.46	410,600	25.66
	Total	47	9,607,000	5,267,000	54.82	1,319,600	25.05
OMC X SST	26 Jun 1986	2	1,489,600	818,100	54.92	3,700	0.45
	29 Aug	2	539,200	141,300	26.21	14,300	10.12
	Total	4	2,028,800	959,400	47.29	18,000	1.88
Corvina	28 Apr 1986	1	500,000	a	a	28,600	5.72[b]
	29 Apr	1	240,000	a	a	600	<0.01[b]
	16 Jun	c	710,000	198,800	28.00	107,000	53.83
	15 Jul	c	1,442,000	1,019,500	70.60	583,800	57.21
	09 Aug	c	678,700	452,000	66.67	155,800	34.46
	20 Aug	c	1,230,600	1,046,000	85.33	543,900	52.00
	Total	c	4,801,300	>2,716,300	>56.57	1,419,700	33.87

[a]Eggs were hatching upon arrival from HHRS and contained a large amount of dead material precluding determination of the number of viable eggs or percent fertilization.
[b]Percent fry return based on total eggs rather than viable eggs.
[c]The number of females participating in tank spawns are unknown. Three females were maintained with three males.

fry survival was consistently near 25%, but SST X OMC hybrid fry survival ranged from 2.5 to 53%.

Fingerling Culture

Fingerling survival for the four groups of fish was highly variable, ranging from 0 to 100%. Survival of spotted seatrout ranged from 26.4 to 100%, orangemouth corvina from 0-100%, and hybrids from 26.7-100%.Mean fingerling size ranged from 25 mm TL and 0.13 g to 69 mm TL and 2.71 g (Table 4), production ranged from 0 to 8.5 kg/ha/day (Table 1).Total numbers of fingerlings of the four groups of fish produced were: spotted seatrout - 68,000; orangemouth corvina - 835,900; SST X OMC hybrids - 42,400 and OMC X SST hybrids - 25,600.

Table 4. Fingerling mean (± SD) weight, total length (TL), standard length (SL), and condition factor (K_{SL}) of spotted seatrout, orangemouth corvina, spotted seatrout female X orangemouth corvina male (SST X OMC) hybrids, and orangemouth corvina female X spotted seatrout male (OMC X SST) hybrid from 22 saltwater pond culture trials.

Trial	Species	Weight (g)	TL (mm)	SL (mm)	K_{SL} [a]
1	SST X OMC	0.84 ± 1.10	47 ± 1.8	35 ± 1.6	1.96
2	SST X OMC	1.14 ± 0.29	52 ± 5.1	41 ± 4.1	1.65
3	Seatrout	0.59 ± 0.07	41 ± 2.2	33 ± 1.7	1.64
4	Seatrout	0.53 ± 0.07	40 ± 1.8	32 ± 1.6	1.62
5	SST X OMC	1.35 ± 0.45	55 ± 6.8	42 ± 6.3	1.82
6	Seatrout	1.37 ± 0.21	53 ± 5.7	43 ± 1.5	1.72
7	Seatrout	0.94 ± 0.16	47 ± 5.2	38 ± 2.5	1.71
8	SST X OMC	1.03 ± 0.17	51 ± 3.1	39 ± 2.7	1.74
9	SST X OMC	0.78 ± 0.12	47 ± 2.7	36 ± 2.1	1.67
10	Seatrout	0.69 ± 0.08	45 ± 2.3	37 ± 2.0	1.36
11	Seatrout	0.54 ± 0.82	42 ± 2.9	34 ± 1.7	1.37
12	Seatrout	0.50 ± 0.07	41 ± 2.1	33 ± 1.8	1.39
13	Corvina	0.69 ± 0.24	43 ± 4.8	31 ± 1.8	2.32
14	Corvina	0.34 ± 0.04	35 ± 3.1	25 ± 2.4	2.17
15	Corvina	0.86 ± 0.28	46 ± 6.9	34 ± 5.2	2.18
16	Corvina	b	b	b	b
17	Corvina	0.24 ± 0.03	33 ± 2.0	24 ± 1.4	1.74
18	Corvina	0.43 ± 0.07	39 ± 2.5	28 ± 1.7	1.96
19	Corvina	0.20 ± 0.03	30 ± 1.5	21 ± 1.0	2.16
20	Corvina	0.13 ± 0.02	25 ± 1.3	19 ± 0.9	1.90
21	OMC X SST	2.71 ± 0.40	69 ± 3.6	53 ± 3.0	1.82
22	OMC X SST	0.16 ± 0.03	27 ± 1.5	20 ± 2.3	2.00

[a]Condition factor=10^5 weight/SL^3

[b]No fish were recovered at harvest.

Survival and production of spotted seatrout and the SST X OMC hybrid were not significantly different in replicated trials, while standard length, total length, weight and condition factor for hybrids were significantly greater than for spotted seatrout (Table 5). Lack of replicated pond trials prevented similar statistical comparison for orangemouth corvina and OMC X SST hybrids. Mean water temperature in ponds ranged from 21-29°C (Table 1), mean salinities from 16-31 o/oo, and mean dissolved oxygen from 2.6-5.0 mg/l. Mean total zooplankton densities during fish culture ranged from 79 ± 59 to 2536 ± 1935 organisms/l (Table 2). The pond culture trial for which no fish were recovered (trial 16) had the longest time between filling and stocking and was one of three ponds with the lowest total zooplankton population. The two ponds with lower zooplankton populations (trials 13 and 14) had the second and fourth lowest survival, and also had the lowest mean temperatures (22 ± 3.9 and 21 ± 5.8°C, respectively).

Table 5. Fingerling mean (± SD) standard length (SL), total length (TL), weight (WT), condition factor (K), survival and production for replicate spotted seatrout, and spotted seatrout X orangemouth corvina hybrid (SST X OMC) pond culture trials. Sample size (*n*) for SL, TL, WT, and K is 100 for each trial, while n for survival and production is 1 for each trial. The *t* statistic for survival was calculated from arcsine transformed survival values. Significance at $P < 0.001$ is indicated by ***.

Group	SST X OMC		SST			SST X OMC		SST		
Trials	8, 9		10, 11, 12			5		6, 7		
	$\bar{x}$	SD	$\bar{x}$	SD	*t* stat.	$\bar{x}$	SD	$\bar{x}$	SD	*t* stat.
SL (mm)	38	3.0	34	2.5	13.3***	42	5.6	41	3.4	1.95 NS
TL (mm)	49	3.6	43	2.9	21.3***	55	6.8	51	3.8	6.10***
WT (g)	0.91	0.19	0.58	0.11	23.9***	1.34	0.44	1.16	0.28	3.80***
K[a]	1.69	0.13	1.42	0.12	24.2***	1.78	0.19	1.70	0.16	3.89***
Survival (%)	37.6	8.2	51.2	13.8	1.18 NS					
Production (kg/ha/day)	1.39	0.03	1.22	0.32	0.70 NS					

[a]$K=10^5$ WT/SL^3

DISCUSSION

Hybrids of spotted seatrout and orangemouth proved to be viable. Both of the hybrids and orangemouth corvina were also successfully cultured in saltwater ponds. Tank spawning of captive broodfish using photoperiod-temperature manipulation to induce spawning appears to be the most efficient method of producing fry. Survival to 2 day old fry of orangemouth corvina eggs spawned through photoperiod-temperature manipulation was more than double the survival rate of strip-spawned spotted seatrout eggs which had the next highest survival rate for eggs to fry. In addition, a smaller number of broodfish are required for tank spawning as the fish can be spawned repeatedly, while the stress involved in hormone induced strip-spawning requires that different fish be used for each attempt. Recent attempts at temperature-photoperiod manipulation to induce spawning of captive spotted seatrout at the MFRS have resulted in production of slightly more than 32 million eggs over 200 days, with a mean survival rate of eggs to fry of 52% (Colura et al. in review). Given the current capability for tank spawning both orangemouth corvina and spotted seatrout, an attempt to produce hybrids by placing appropriately conditioned individuals of opposite sexes and species in a common tank might result in successful interspecific spawning. However, strip-spawning is currently the only proven method of producing the hybrids.

Fingerling production of orangemouth corvina and the two hybrids exceeded initial pond culture results reported for other marine fishes including red drum (*Sciaenops ocellatus*) (20 ± 20.4% survival and 0.96 ± 0.87 kg/ha/day production) and snook (*Centropomus undecimalis*) (12 ± 11.67% survival and 0.13 ± 0.1 kg/ha/

day production) (Colura et al. 1976, Maciorowski et al. 1986). Spotted seatrout fingerling survival in the present study, 51%, was approximately six times the 8.4% survival reported by Porter and Maciorowski (1984), although overall production, 1.06 kg/ha/day, was approximately half of their reported 2.1 kg/ha/day. However, spotted seatrout fry stocking rates in this study were less than 15% of those used by Porter and Maciorowski (1984). Improved spotted seatrout fingerling survival, and the relatively high fingerling production for the first pond culture attempts with orangemouth corvina and the two hybrids, may be attributable to a combination of improved spawning techniques (Colura et al. 1988, 1990; Prentice et al. 1989), pond management strategies designed to increase zooplankton forage (Braschler 1974, Geiger 1983*a*, 1983*b*) and lower pond stocking densities.

The importance of predictable spawning to pond culture of spotted seatrout has been previously discussed (Porter and Maciorowski 1984). Ideally, fertilized culture ponds should be stocked with fry when appropriately sized zooplankton are undergoing peak reproduction (Geiger 1983*a*, 1983*b*). Accordingly, spawning should be synchronized with pond preparation to allow maximum zooplankton development prior to fry stocking. Pond preparation should precede spawning and fry stocking by approximately 3 weeks to ensure appropriate zooplankton are available for newly feeding fry (Porter and Maciorowski 1984). In past studies (Colura et al. 1976, Porter and Maciorowski 1984) spotted seatrout spawning predictability has been marginal. Improved spawning predictability in the present investigation was a direct result of related spotted seatrout spawning research which delineated criteria for evaluating eligibility of females for strip-spawning and identified hormone levels necessary to induce ovulation prior to strip-spawning (Colura et al. 1988, 1990). Similarly, spawning methods for orangemouth corvina have only recently been sufficiently refined to allow reasonable predictability. The development of a temperature-photoperiod regime combined with a spawn-inducing technique (abrupt temperature drop) has made the production of large numbers of orangemouth corvina embryos from captive broodstock possible (Prentice et al. 1989).

Differences in size of spotted seatrout and the SST X OMC hybrids from replicate ponds may be attributable to the nominally greater survival of spotted seatrout, as size is inversely correlated to the number of fish harvested from a pond (McCarty et al. 1986). Larger size of the SST X OMC hybrids compared to spotted seatrout might also be due to increased capability for growth imparted either by the orangemouth corvina parent or due to hybrid vigor (Avers 1984).

Survival of less than 10% was apparently due to lack of forage as the two ponds in this category (trials 16 and 13) had the lowest zooplankton densities of any pond culture trial (Table 2). Survival greater than 100% reflects the variability present in fry enumeration procedures. Fry enumeration relied on the assumption that fry were evenly distributed within the fry collection chamber. Uneven fry distribution in the fry collection chamber would cause errors in fry estimates and affect the number of fry placed in transport bags. Matlock et al. (1986) reported red drum fry estimation techniques overestimate the actual number of fry stocked in ponds. Results of trials 3, 20 and 22 indicate that on at least three occasions the number of spotted seatrout,

orangemouth corvina, or hybrid fry stocked was underestimated.

Successful production of fingerling orangemouth corvina and the two hybrids indicates the fish are amenable to pond culture and could be produced in large numbers to satisfy the requirements of a stocking program. An effort to increase the efficiency of hybrid fry production would be necessary to support a large scale stocking program or a culture venture. Size comparisons of the SST X OMC hybrid and spotted seatrout indicated the hybrid may have a growth advantage over the spotted seatrout parent, which would make the hybrid a more suitable candidate for commercial aquaculture. However, additional research with the hybrids to determine their reproductive status, temperature and salinity requirements, and growth capacity in captivity should be conducted before the fish are considered for use in fisheries management or aquaculture.

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NOTES ON A MASS STRANDING OF BAIRD'S BEAKED WHALES IN THE GULF OF CALIFORNIA, MEXICO

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A group of four male and three female Baird's beaked whales (*Berardius bairdii*) stranded themselves on 2 July 1986 at El Mogote, near La Paz City, B. C. S., Mexico. The stranding was the southernmost record of this species on the eastern North Pacific coast, the first for the Gulf of California, and the first known mass stranding for this species. Animals ranged between 9.03-11.35 m body length and 13-42 years of age according to cemental layers. Anterior teeth eruption seems to occur after 9 m of length, near the length of the attainment of sexual maturity.

INTRODUCTION

The Baird's beaked whale (*Berardius bairdii*) is endemic to the north Pacific (Leatherwood et al. 1982, Minasian et al. 1984). It has been found on the North American coast from Alaska to southern California (Pike 1953, Slipp and Wilke 1953, Rice 1963). The records from the eastern Pacific show that the whales are more frequent inshore from July to October (Rice 1974).

Important studies of the biology of Baird's beaked whales in Japanese waters have focused on morphology, sexual maturity, catch records, age determination, and growth (Omura et al. 1955, Nishiwaki and Oguro 1971, McCann 1975, Kasuya 1977, 1986). They mostly inhabit deep waters (Nishiwaki and Oguro 1971). The stranding of a school Baird's beaked whales at Bahia de La Paz, Baja California Sur, in the southwestern Gulf of California on 2 July 1986, was a rare opportunity to study the species.

METHODS

Only body length was measured because advanced decomposition precluded other measurements. Body length was the distance of a parallel line from the tip of the snout to the middle point of the flukes. The sex was evident in the males because the penis was extruded and visible. In animals where the penis was not evident, a search for the two slits of the mammae on either side of the genital opening determined the sex.

One anterior mandibular tooth from three individuals and two from three others, were extracted. No teeth from one specimen, whose teeth had not yet erupted, were extracted. Each tooth was labeled, cleaned of tissue, dried, and weighed. Three measurements were taken from each tooth; the height from the base to the apex, the

length of the base, and the exposed length of the tooth (ELT), defined as the vertical length from the tooth apex to the anterior border separating the exposed area of the gum on the side facing the tongue. One tooth from each animal was sagittally sectioned with a fretsaw. A cut face from each tooth was polished with a series of three wet sandpapers, from coarse to fine grain, until the surface was smooth. The teeth were then immersed in a 10% solution of formic acid for 12 h. This procedure, has been successfully used for aging California sea lion teeth (Aurioles-Gamboa 1988), by counting layers directly through a stereoscope. The area of the tooth suggested by Kasuya (1977) was used to count the number of teeth layers. Because saggital sections were obtained, two areas for counting the cemental layers on each tooth were available, and at least three counts were made in order to obtain a mean value. The long-cycle layers that correspond to annual periods for this species and other odontocetes and pinnipeds (Oshumi et al. 1963, Klevezal and Kleinenberg 1967, Kasuya 1977), were more evident than the short-cycle layers also reported by Kasuya (1977) in teeth of *B. bairdii*. Hence, for the present study only the number of the long-cycle layers were recorded.

RESULTS

The whales were reported stranded on 4 July 1986 at "El Mogote", a sandy extension that protects La Paz from the direct waves of Bahia de La Paz (Fig. 1). This sandy formation constitutes the southern edge of the bay, where strandings of marine mammals occur frequently (Gilmore 1957, Norris and Dohl 1980, Aurioles-Gamboa unpubl. data). We visited the site where seven individuals were found half submerged. The stranding occurred during the night between 1 and 2 July.

Six animals were dark in color and in an advanced stage of decay. Day air temperature was above 37°C, causing rapid decomposition. The best preserved individual (specimen 7, Table 1), had a dark gray skin, not black as the other animals. The black color of most of the individuals was likely due to the effect of the sun burning or a postmortem color change. All the animals exhibited many scratches on the back but and ventral sides of the body, except for specimen 2 (Table 1).

The animals were separated from one another by short distances. Two animals, specimens 5 and 6, were in body contact (Fig. 1, Table 1). Specimens 3, 4, and 5 were separated by 1-2 m, and specimen 1, was 5 m from the closest animal. Only specimen 7 (Fig. 1, Table 1), was relatively far away from the group, at a distance around 30 m.

The external morphology of the animals is summarized as follows; a prominent melon and an elongated snout with the mandible protruding outward several centimeters distant from the tip of the upper jaw. An anterior pair of teeth was protruding from the gum, and in some cases an extra pair behind the first one. The smallest individual (specimen 2) had teeth that were not yet erupted. The largest animal (specimen 6), had only the right anterior tooth but the gum cavity in the mandible showed that the other had been lost. This condition has been reported occasionally in males (McCann 1975).

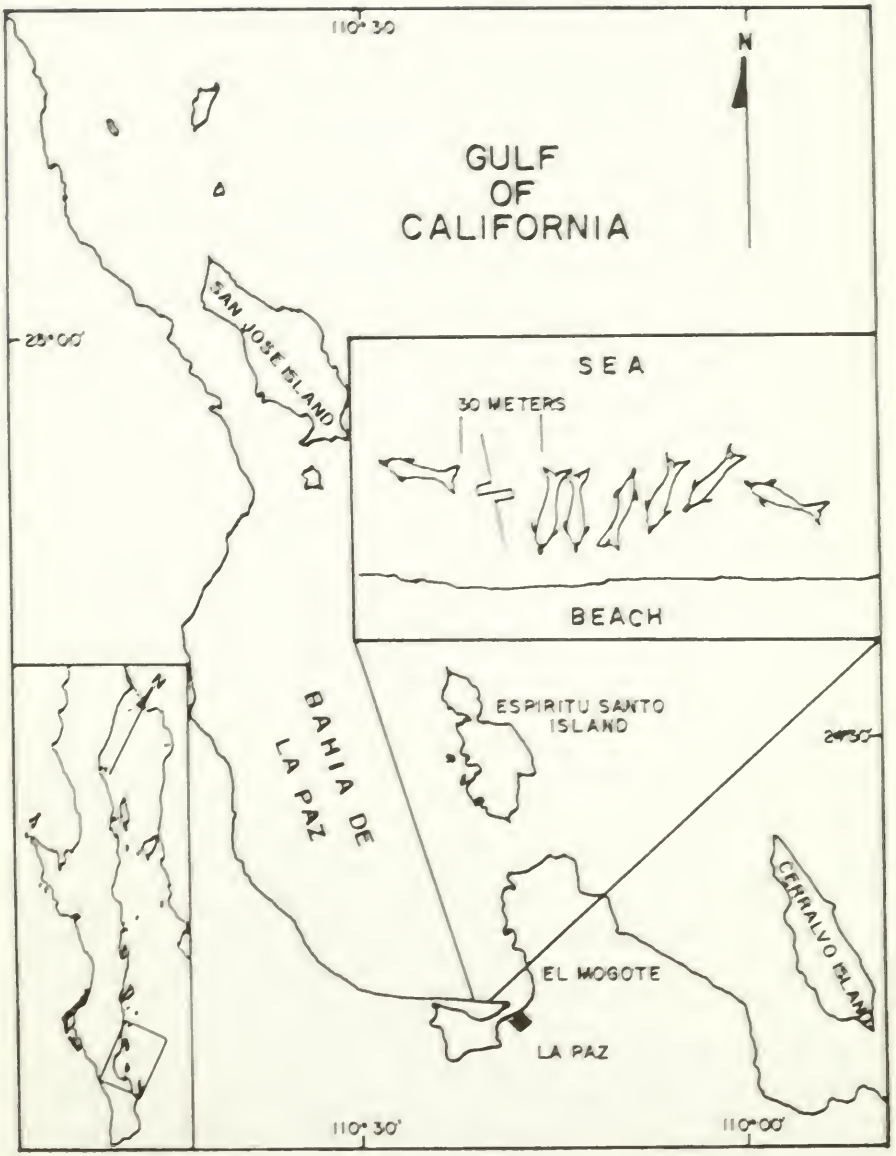

Figure 1. Location of the seven Baird's beaked whale school stranded in the Gulf of California. The position of the animals on the stranding site is shown in the box. Specimen 1 is the first on the right, and the following numbers (2-7) were respectively assigned to the next specimens.

Table 1. Morphological data from seven Baird's beaked whales (*Berardius bairdii*) stranded in Bahia de La Paz, Gulf of California.

			Tooth measurements					
No.	Sex	Body length (m)	Tooth growth layers		Tooth weight (g)	Height cm	Base length cm	E.L.T. cm
1	M	11.1	29	R	281.1	9.2	8.5	3.0
				L	314.2	9.3	8.7	3.2
2	F	9.05			tooth not extracted			
3	M	10.83	25	R	286.1	9.8	7.2	4.5
				L	268.4	9.6	7.2	4.1
4	F	9.85	13	L	175.7	8.7	8.0	0.5
5	M	10.25	20	R	243.5	9.5	7.8	3.1
				L	251.5	9.5	8.2	2.9
6	M	11.35	42	R	314.7	9.6	9.4	5.0
7	F	10.55	17	L	215.0	9.6	7.8	2.8

The dentition is diagnostic for *Berardius* (McCann 1975). The species was assumed to be *B. bairdii* because the other longener, *B. arnuxii* (Arnoux's beaked whale) is reported from the southern hemisphere, and does not exceed 9.9 m (McCann 1975, Minasian et al. 1984), whereas the body length range of the stranded animals was 9.05-11.35 m (Table 1).

Sex and Age Composition of the Stranded School

The group was composed of 3 females and 4 males. Two females (specimens 2 and 4), were probably immature, and the 5 remaining animals were in the size range at which sexual maturity is attained (9.7-10 m males, 10.1-10.4 m females, Omura et al. 1955). Data on body length, age (cemental layers counts) and sex are shown in Table 1. The ages according to the counting of cemental layers were in between 13-42, but the age of the smallest was not ascertained. The size and the unerupted teeth indicate this animal was the youngest (specimen 2, Table 1).

Body Length, Cemental Layers, and Eruption of the Anterior Teeth

The whales had different degrees of tooth eruption and that the longer the animal, the more exposed the anterior teeth. Omura et al. (1955), observed that the anterior teeth are erupted in all mature animals, and usually concealed in the gum in immature individuals. Body length of the animals was plotted against the exposed length of teeth (ELT, Table 1). The ELT value from individual 2, was computed as zero, since the anterior teeth had not erupted. The body length at which the line intersects the x-axis is in between 9 and 10 m, which suggests that tooth eruption occurs several years after birth (Fig. 2).

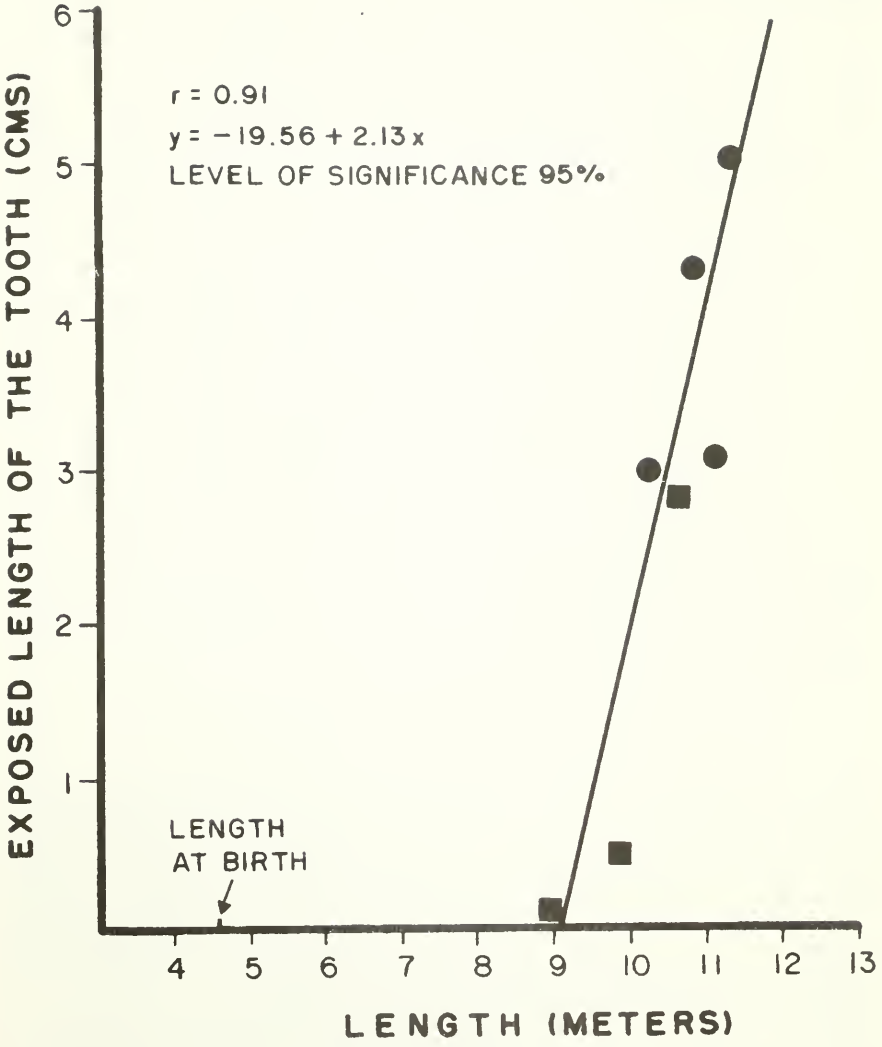

Figure 2. Body length related to Exposed Length of the Teeth (ELT) of animals in the stranded school. Circles = males; squares = females.

One specimen from British Columbia, was an immature female with a body length of 8.8 m that had unerupted anterior teeth (Pike 1953). A 9.6 m female *B. bairdii* stranded in northern California had teeth still buried in the gum (Sullivan and Houck 1979).

Cemental Layers and Body Length in North Pacific Individuals

The cemental layers counts were plotted against body length and included in the growth curve calculated from animals caught off Japan by Kasuya (1977). The body length-age data from the stranded animals in the Gulf of California fit well to this growth curve. The values, however, fit even better to a more recent plot for males and females provided by Kasuya, Brownell and Balcomb (1988) in which more data from specimens caught recently were added. If specimen 2 (Table 1), whose teeth were not extracted, is aged according with its body length in the growth curve for females from Kasuya et al. (1988) its age would be between 5 and 10 years.

DISCUSSION

The stranding of Baird's beaked whales inside the Gulf of California, was the southernmost record for the species for the eastern North Pacific coast, the first record in the Gulf of California and the first of two known mass stranding for this species. Kasuya et al. (1988) reported a mass stranding in the summer of 1987 consisting of 4 individuals at Moroiso, Kanagawa Prefecture in Japan. Apparently single strandings are more frequent along the coast from Alaska, the Aleutian Islands, and Siberia (Mead et al. 1988).

Rice (1974) reported the range of the *B. bairdii* in the eastern Pacific from the Bering sea to southern California (lat 32°30' N). Leatherwood et al. (1982), reported the southern limit as Baja California (lat 28°N). The present record set the range to lat 24°11' N, long 110°22' W, but the southern limit should be considered for now, as Cabo San Lucas or the southern Gulf of California.

The present record of *B. bairdii* in the Gulf of California for the month of July, agrees with other sightings or single strandings in the eastern Pacific. These occur from June to October (Slipp and Wilke 1953, Pike 1953, Rice 1963, Sullivan and Houck 1979, Leatherwood et al. 1982). The majority of catches off British Columbia occur in August, but California catches suggest two peaks of abundance, in July and October (Rice 1974).

This species has been reported in Japanese waters during the whaling season, which start in May and finished in November (Omura et al. 1955, Nishiwaki and Oguro 1971), suggesting that the Baird's beaked whales have seasonal occurrences near shore on both sides of the North Pacific during summer and fall.

Based on the data presented here, the anterior teeth seem to erupt after the animal reaches 9 m of body length (Fig. 2). The data suggest that there is a period between the length at birth (4.5 m, Kasuya 1977) and the 9 m of length, during which the teeth are not erupted. The difference of about 4.5 m, represented several years of growth. It is generally accepted that the reduced dentition in ziphids serves little utility as a feeding mechanism. Omura et al. (1955), reported that the anterior teeth are always erupted on mature animals, and usually concealed beneath the gum in immature whales.

Sexual maturity occurs for males and females near 9.7-10.4 m (Omura et al. 1955). This range is close to the length here estimated (Fig. 2), at which the eruption of the anterior teeth occurs. Thus teeth erupt at about the age of sexual maturity and are probably used during courtship and breeding.

The present stranding occurred on the southern area of the Bahia de La Paz, where other mass strandings have been reported involving sperm whales (*Physeter macrocephalus*) (Gilmore 1957) and pilot whales (*Globicephala macrorrhyncus*) in 1957, 1966 and 1989 (Norris and Dohl 1980, R. De la Parra and J. Urban, pers. comm.), and many single strandings involving, Cuvier's beaked whales (*Ziphius cavirostris*), dwarf sperm whales (*Kogia simus*), melon headed whale (*Peponocephala electra*), fin whale (*Balaenoptera physalus*), Bryde's whale (*Balaenoptera edeni*), and other more common species such as bottlenose (*Tursiops truncatus*), rough-toothed (*Steno bredanensis*), and common dolphins (*Delphinus delphis*). The shallow topography of the southern part of the Bahia de La Paz (< 50 m), probably contributed to the stranding of these usually pelagic species.

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A COMPARISON OF RUMEN CILIATES IN BLACK-TAILED DEER FROM CALIFORNIA AND HAWAII

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Ciliated protozoa were used to indicate changes in the rumen microbial population of two groups of concentrate-selecting black-tailed deer (*Odocoileus hemionus columbianus*) after 25 years of total separation in Tehama County, California and Kauai, Hawaii respectively. The two groups showed no significant difference in rumen species composition.

INTRODUCTION

Black-tailed deer (*Odocoileus hemionus columbianus*), belong to the group of fruit and dicotyledon foliage selectors (Hofmann 1973). A large herd inhabits the Tehama County area (hereafter Tehama) of California and forages primarily on browse except in the fall, when forbs and sprouting annual grasses become the main diet (Leach and Hiehle 1957). Between 1961 and 1966, two groups of breeding does and bucks, totalling 31 and 9 animals respectively, were translocated to the brushy habitat of the western Kauai area of Hawaii (Telfer 1988). On Kauai, browse, consisting chiefly of passionflower vine (*Passiflora edulis*) and lush exotic plants constituted the diet except in autumn when this changed mainly to fruits of guava (*Psidium guajava*) and passionflower vine (Telfer 1988).

The deer on Kauia were protected from hunting for eight years after their initial release. Controlled deer hunting was first allowed in October 1969, and continued in this manner annually during September and October (Telfer 1988). Owing to dense vegetation, rough topography, and limited manpower, quantitative data of the deer population status was unavailable. Thus, Telfer (1988) not only made subjective evaluations based on track and pellet abundance, and browse use, but also an estimate based on two observations. First, of 53 does and fawns observed between July and December from 1984 to 1987, 23 (43%) were fawns (Telfer 1988); and second, the annual harvest yielded a ratio of yearlings to adults of 1:1.55 (Telfer 1988). These

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observations indicated a high potential reproductive rate of approximately 50% annually (Telfer 1988). Further, Kauai yearling does bred successfully (Telfer 1988), whereas successful breeding in most Tehama does occurs at 1.5 years of age and the birth of their first fawns at two years (J. G. Kie, pers. comm.). Nevertheless, since 1969 the Kauai deer population declined progressively until the herd stabilized at approximately 350 animals west of Waimea Canyon (Telfer 1988). Observations attributed the decline to poaching and hunting-dog depredation.

Groups of seven deer shot in Tehama and six in Kauai in 1989 were used for the present study, in which the ciliated protozoa were used to indicate any changes in the rumen microbial population of the two groups.

MATERIALS AND METHODS

The gastrointestinal tract was excised immediately after the deer was shot. A slit was made in the reticulorumen wall. The digesta was mixed by hand, and filtered through two layers of cheese cloth. The filtered rumen fluid was preserved with 10% formalin for examination by light microscopy of unstained wet-mounted slide preparations at magnifications of x400, and x1000 using an oil immersion objective. Protozoa characterization was carried out with reference to Corliss (1979), Dehority (1985), Hsiung (1930), and Dogiel (1925*b*). Different ciliate species were counted at x400 magnification and converted to a percentage of a total in excess of 200 individuals (Van Hoven et al. 1987). The *T*-test was used for statistical evaluations.

RESULTS AND DISCUSSION

No significant difference ($P < 0.01$) existed in the mean percentage composition of rumen ciliate species between the Tehama and Kauai groups of deer (Table 1). Because diet is the major factor determining rumen microbial composition (Dehority and Orpin 1988), this similarity in rumen protozoa indicated that the chemical composition of the diet selected by Kauai deer was similar to that selected by Tehama deer despite the widely different plant species from which selection was made. Extensive studies on East African concentrate-selecting antelopes showed that these animals were indeed very highly selective for only lush dicotyledonous foliage from a wide variety of plant species (Hofmann 1973, Hoppe 1984). Thus, the black-tailed deer could have managed to select diets of similar chemical composition despite the great differences in habitats and browse species. The ciliates consisted of nine *Entodinium* species, and two *Isotricha* species found in only three Tehama deer (Table 1). There was a marked predominance in both Tehama and Kauai deer of *E. dubardi*, mean composition of 48 and 50% respectively, and *E. exiquum*, mean of 34 and 32%, respectively. The predominance of *Entodinia* is typified among the fruit and foliage selectors by Kirk's dik-dik (*Madoqua kirkii*) of East Africa (Hofmann 1973). Hoppe (1984), using gas production by fresh rumen digesta, showed that fermentation rates of dik-dik were three times that of large grazers (Hoppe 1984). This implied a rapid rate of passage of digesta out of the reticulorumen. Under such

Table 1. Mean percentage composition of rumen ciliates from black-tailed deer in Tehama[1] and Kauai[2].

Ciliate	Tehama $\bar{x}$	Tehama range	Kauai $\bar{x}$	Kauai range
Entodinium ovinium	1	(1-3)	0	0
E. dubardi	48	(29-60)	50	(43-59)
E. simplex	3	(0-8)	5	(2-7)
E. longinucleatum	1	(0-2)	3	(0-5)
E. exiguum	34	(21-62)	32	(24-36)
E. nanellum	1	(0-3)	4	(0-9)
E. loboso-spinosum	4	(0-15)	1	(0-4)
E. furca dilobum	1	(0-2)	0	0
E. triacum	6	(0-21)	6	(2-11)
Isotricha prostoma	1	(0-1)	0	0
I. intestinalis	2	(0-4)	0	0

[1]Tehama group comprised of samples C1-C7.
[2]Kauai group comprised of samples K107, K151, K152, K153, K154, and K156.

conditions, only the rapid multiplying *Entodium* could maintain themselves in the rumen (Van Hoven 1984). This likely applies also to black-tailed deer. The colder winters and drier summers of Tehama compared to Kauai (Telfer 1988) would slow plant growth, resulting in a decrease in energy available to Tehama deer, consequently a marked decrease in their rumen microbial concentration (Hobson et al. 1976). Since the anaerobic bacteria and ciliates account for most rumen fermentative activity, a decrease in their concentrations would add to the decrease in nutritional end products. Conversely, plant growth and hence energy intake by Kauai deer would be much more consistent throughout the year, resulting in good nutritional status of these animals. This was demonstrated by the excellent rate of weight gain, higher carcass weight, and better reproductive health of Kauai deer compared to Tehama deer (Telfer 1988). It also supports Telfer's (1988) estimate that the habitat west of Waimea Canyon of Kauai could support 2,000 deer with adequate management and protection against poachers and hunting-dogs, instead of the present static total Kauai complement of 350 deer (Telfer 1988).

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SWALLOW MORTALITY DURING THE "MARCH MIRACLE" IN CALIFORNIA

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Much of California has been subject to drought conditions since 1986. However, during March 1991, a period of heavy rains fell in many parts of the State which was popularly referred to as the "March Miracle". An interesting phenomenon of swallow mortalities accompanied the rains and attendant cool weather.

The Pesticide Investigations Unit of the California Department of Fish and Game (CDFG) accepts animal specimens to determine if a pesticide was the cause of death. We were asked to determine the cause of death of approximately 20 cliff swallows *(Hirundo pyrrhonta)* that died on 28 March 1991 in the Mendota region of the San Joaquin Valley. However, after discussions with local CDFG biologists, it was concluded that weather was the cause of death and no further action was taken at that time.

Thirty cliff swallows were found dead at a nest site at Pismo Beach, on the central coast of California on 29 March. Another nearby colony was apparently doing well. No pesticide exposure could be suggested from the circumstances at the site.

A more detailed investigation seemed justified at this point, because these two incidents had occurred within a matter of days. Birds at the Mendota site had been seen dying inside and outside a sugar processing plant. The occurrence of the swallows inside the building was unusual. At Pismo Beach, several dead birds were found huddled together in the previous year's mud nest, and other birds were found dead on the ground.

We contacted the U.S. Fish and Wildlife Service's (USFWS) National Wildlife Health Research Center at Madison, Wisconsin to discuss these two incidents. Center personnel responded that they were interested because they were working on a similar case from Irvine, in Southern California, where approximately 60 cliff swallows had died. Only two swallows were available for necropsy as community maintenance personnel had disposed of the rest. These were examined at the USFWS Madison Lab but the cause of death was not determined. The USFWS had also received a report of 10-20 dead swallows at the Tijuana Slough in San Diego County, but unfortunately no birds were suitable for analysis. They also received a report of exhausted birds which could be easily approached at Pt. Mugu, Ventura County. Both the Tijuana Slough and Pt. Mugu reports came in on 1 April. Similar reports started becoming more numerous as additional inquiries were made.

R. Bruggeman, CDFG, Fresno (pers. comm.) suggested die-off's in the spring were not unusual. He referred us to the Gray Lodge Wildlife Area, approximately 100 km north of Sacramento, as another locality where deaths had occurred. We were

told by wildlife area staff that several species of swallows had died there. Birds were seen huddled together inside structures. Swallow deaths were common enough that television reporters were making inquiries. This led to another report that approximately 100 tree swallows *(Tachycineta bicolor)* had died in early March at a house under construction in southern Tehama County, about 50 km north of Gray Lodge. Two weakened birds were taken to a local animal care center where they recovered and were released.

The rains subsided after this period, but another loss occurred. Approximately 400 cliff swallows, four tree swallows, and one bank swallow *(Riparia riparia)* were found dead on 11 April, on a gravel levee top road along the Sacramento Deep Water Ship Channel adjacent to Sacramento. About half the animals had been run over by one or more vehicles. Workers at the site said the birds were reluctant to fly, and that drivers had to travel slowly to allow the birds to disperse.

The confirmed swallow losses were associated with the uncommon weather patterns that had occurred in March.The Mendota loss reported on 28 March was concurrent with rain and cool weather.The weather conditions at Los Banos, approximately 30 km north, were cooler and wetter than normal (NOAA 1991). Low temperatures for the period ranged from approximately 8°C on 24 March to 6°C on 29 March. The lowest temperature in the period was 2°C. Rainfall occurred every day from 24-29 March, with the total rainfall for the month at an above average 8.5 cm.

At Pismo Beach, approximately 43 cm of rain fell resulting in the wettest March on record. Lows were from 3°C to 6°C from 26-28 March. Rain fell all three days and ranged from 0.5 cm to 3 cm per day during the period.

The Sacramento Deepwater Channel area was windy at the time the swallows were killed there. Winds to 33 kph out of the northwest were reported at Sacramento Executive Airport on 10 April. NW winds to 51 kph were reported on 11 April. Reports from citizens working in the area and driving on the levee road indicated that the birds were reluctant to fly in the wind.

Necropsies of the swallows from the deepwater channel levee indicated some body fat was present. Other necropsies by the pesticide unit and by the USFWS could not determine the specific cause of death. However, these findings did not rule out weather-related causes.

The world literature contains many reports of weather-related mortalities of adult swallows. Kimball (1889) reported a mortality among eave swallows (cliff swallows) in Illinois similar to that experienced here. He reported that dead birds were found in nests. This was also seen in the 1991 Pismo Beach occurrence. Kimball (1889) attributed the loss to cool weather depressing insect populations after a warm period. Other species also seemed to have been affected. Edson (1943) reported that violet-green swallows *(Tachycineta thalassina)* were killed in Washington in March 1936, when snow fell and temperatures declined to 23°F (-5°C). Allen and Nice (1952) cited other authors in which several incidents of martin *(Progne subis)* mortalities were due to unfavorable weather. Brockhuysen (1953) examined birds which died under adverse weather conditions in South Africa. Martins *(Delichon urbica)*, *(Ptyonoprogne fuligula)*; swallows *(Hirundo rustica)*; *(H. abyssinica)*; and swifts *(Apus barbatus)*

were involved. Mayhew (1958) described the effects of temperature on the arrival of swallows in the Sacramento area, and suggested cooler weather seemed to delay arrival. His arrival dates were similar to the dates of the death of the birds investigated here. Skeal and Skeal (1970) reported the circumstances surrounding the deaths of *Hirundinidae*, again in South Africa in the Transvaal and Orange Free State during unusual cold wet weather. Robins (1970) cited other authors describing behavior similar to that reported to the pesticide unit. Birds responding to cold weather huddled together in protected areas. Finally, Henny et al. (1982) reported that DDE was not implicated in the death of cliff swallows. They stated a "cold snap" was probably the cause of death in May 1980.

The "March Miracle" was certainly a welcomed climatological event. Some swallows, however, were adversely affected by the cool wet weather and mortalities, extending over approximately 800 km through California, were reported.

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Several individuals provided reports of swallow mortalities. CDFG Wildlife Habitat Supervisor II R. J. Huddleston reported the first loss near Mendota. CDFG Wildlife Biologist J. Lidberg reported the loss at Pismo Beach. Discussions with C. Quist at the USFWS center in Wisconsin lead to detailed reports from D. Audit of the USFWS regarding losses at Irvine, Tijuana Slough, and Pt. Mugu. D. McCraken at the CDFG Gray Lodge Wildlife Area reported recurrent deaths there. W. Ginno provided information on the bird loss at his construction site in Tehama County. The large loss at the Sacramento Deep Water Ship Channel was investigated in cooperation with L. Owens, USFWS Special Agent.

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OBSERVATION OF BLACK BEAR (*URSUS AMERICANUS*) PREDATION ON COLUMBIAN BLACK-TAILED DEER (*ODOCOILEUS HEMIONUS COLUMBIANUS*)

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Mule deer, including the black-tailed subspecies, are widely recognized as prey for a number of predators. A comprehensive overview of case studies evaluating predator impacts on deer populations is provided by Connolly (1981). However, few documented cases exist of eyewitness accounts of natural mortality between deer and predators. Brown et al. (1988) gave an example of mountain lion mortality as a result of an encounter with an elk (*Cervus elaphus*). Others have published these types of interactions between individuals (Hornocker 1970) but documented accounts are very rare. Neal (1990) gave an overview of mountain lion predation on the North Kings Deer Herd. Leopold et al. (1951) discussed the importance of black bear predation on the Jawbone mule deer herd based on scat analysis. They demonstrated that black bears consumed substantial quantities of deer. In their discussion, Leopold et al. (1951) recognized the importance of carrion as a source of bear food but also acknowledged that significant numbers of fawns were most likely killed.

Little information is documented regarding black bears in the redwood forest type. Giusti and Schmidt (1988) presented an overview of black bear biology in the redwood forest that included cited studies of bear food habits from various forest types throughout California.

We present an eyewitness account of the killing of a mature, male black-tailed deer by a black bear. The incident took place in the Hunter Creek drainage of the Klamath River in Del Norte County, California. The area is predominated by redwood forest habitat (Mayer and Laudenslayer 1988) being managed for commercial timber production. The incident occurred on 19 October 1991, just prior to sunset, at approximately 1700 hours. The weather was clear with good visibility.

A bear (approximately 150 kg) was observed by SLC browsing along a logging road, adjacent to a 40 acre clear-cut unit, at a distance of approximately 250 m. SLC quietly stood up to leave to avoid disturbing the bear. SLC heard a loud, crashing noise, approximately 300 m from his location, and looked toward the top of the ridge.

The sound was made by a mature, male black-tailed deer (3x3 antler spread, approximately 55-65 kg) being chased by a second black bear (approximately 100 kg) through the brush covered, clear-cut unit. After a 5 to 7 seconds pursuit, the deer

was knocked over by the bear. Following a struggle the deer raised to its feet and was knocked down a second time.

The deer again regained its footing and ran downhill toward the observer. At this time the deer showed no visible wounds. The chase continued with the deer using a stotting gait and the bear running through, not over, the brush. Over the elapse of a few seconds the deer was knocked down two more times. By this fourth attack, the deer was bleeding heavily from the chest area and was showing signs of stress and fatigue. It no longer stotted over the brush, but rather ran through it.

By this time, the two animals were less than 50 m from the observer. The bear once again knocked the deer down. Following this fifth attack, the deer never again regained its footing. Both animals were now in heavy brush and the deer was obscured from sight. Immediately following the final attack the bear could be heard tearing at the flesh and breaking bones on the deer. From the time the animals were first observed to the time the bear began to feed on the deer, the incident took less than one minute.

The first bear was observed still standing and watching the incident following the attack. The second bear, now feeding on the deer, apparently detected the first bear or the observer and began to produce long, and very loud roars that lasted 4-5 seconds. It did this three consecutive times. These roars caused the first bear to immediately run from its location away from the incident.

Following the attack, the observer, SLC, prepared to again leave the area. His presence was detected by the feeding bear and the bear immediately made two false charges. While charging, the animal made sounds similar to the roars following the kill. The bear approached within 35 m of the observer and showed no intention of retreating. At that time the observer left the area.

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